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BACTERICIDAL EFFICACY OF SANITIZERS PRODUCED BY COMMERCIAL WATER TREATMENT GENERATORS

by
Edmund M. Powers*
Claire Lee
Joshua Magnone
and
Chad Haering

***Edmund M. Powers Company**
Framingham, MA 01701

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U.S. Army Natick Soldier Research, Development and Engineering Center
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*Edmund M.Powers Company
14 Simpson Drive
Framingham, MA 01701

14. ABSTRACT

Five commercial water sanitizer generators were tested, by the U.S. Army Natick Soldier Research, Development and Engineering Center (NSRDEC), for bactericidal efficacy of their sanitizer on stainless steel surfaces. Sanitizers generated were Acid Electrolyzed Water (AEW), hydrogen peroxide, mixed oxidants, and ozone. Manually produced chlorine dioxide was also tested. Surfaces were inoculated with three strain cocktails of *S. aureus* and *E.coli*, dried and then immersed in a solution of each sanitizer. Efficacy was determined by achieving a 5 log reduction within 5 min. AEW concentrations of 80 -100 ppm free available chlorine (FAC) and chlorine dioxide at 100 ppm were effective with both adherent and non-adherent cells. Such generators would provide the Army Mobile Kitchen Trailer (MKT), the Assault Kitchen (AK), and the Containerized Kitchen (CK) with the means to produce an effective sanitizing solution in the field without re-supply. They would also eliminate the logistical problems and hazards entailed with the purchase and shipment of chemicals throughout the military supply system. The generators will require electrical power and a potable water supply.

15. SUBJECT TERMS

WATER	KITCHENS	GENERATORS	FOOD DISPENSING	WATER TREATMENT
BACTERIA	SANITIZERS	MOBILE KITCHENS	CONTAMINATION	ESCHERICHIA COLI
SURFACES	SANITATION	STAINLESS STEEL	STAPHYLOCOCCUS AUREUS	

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PREFACE

This report describes a study to evaluate the bactericidal efficacy of five commercial-off-the-shelf sanitizer generators for producing sanitizer solutions for use on food preparation surfaces in the field. The study was performed by the U.S. Army Natick Soldier Research, Development and Engineering Center (NSRDEC) in response to the Technology Thrust Proposal entitled "Sanitizer Generator", under Program Element Number AH99 6.2 JV2020. The work was initiated in October 2006 and completed in September 2008. The Project Officer was Chad Haering, Combat Feeding Directorate, Equipment and Energy Technology Team.

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The authors and the Performance Optimization Research Team, and the Food Safety and Defense Team, thank Donald Pickard, Leader of the Equipment & Energy Technology Team for supporting the project. We also thank Betty Davis, PORT team leader for her encouragement and support. Our thanks to Patrick Marek also, for his help in computer formatting some of the early tables.

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BACTERICIDAL EFFICACY OF SANITIZERS PRODUCED BY COMMERCIAL WATER TREATMENT GENERATORS

1. INTRODUCTION

This report documents an evaluation of five commercial water sanitizer solution generators for bactericidal efficacy on inanimate, nonporous food contact surfaces. This study was performed by the U.S. Army Natick Soldier Research, Development and Engineering Center (NSRDEC), in support of the Joint Technical Services (JTS) Committee, between October 2006 and September 2008.

Not all Manufacturers of the subject generators make claims for the sanitizing of hard surfaces by the solutions produced, since the generated solutions are intended for water purification. However, Acid Electrolyzed Water (AEW) [1], chlorine dioxide [2, 3, 4], hydrogen peroxide [5], and ozone [6,7] have been used in food preparation facilities to sanitize equipment and prevent spoilage of fruits, vegetables, meat, and fish.

A need was identified to provide the Army Mobile Kitchen Trailer (MKT), Assault Kitchen (AK), and Containerized Kitchen (CK) with the means to produce an effective sanitizing solution in the field. Such a system would enable the mobile kitchens to maintain sanitation without re-supply. It would also eliminate the logistical problems and hazards entailed with the purchase and shipment of chemicals throughout the military supply system. Such a capability would complement a waterless sanitation system already developed for the MKT to be used when water is not available in the field for sanitation [8, 9].

The objective of this study was to determine the bactericidal efficacy of sanitizer solutions, produced by commercial off-the-shelf generators, on a hard nonporous stainless steel surface. The surface material used in the study was selected to simulate the stainless steel surfaces common to the field kitchens. Each sanitizer solution was tested at the concentration produced by the generators.

2. MATERIALS AND METHODS

2.1 Sanitizer Generators

Five sanitizer generators were tested. They are listed and briefly described in Table 1. All five produce their sanitizers through the electrolysis of ordinary tap water in specially designed reactors or electrolytic cells. The ElectroCide process (Electrolyzer Corporation and Hoshizaki Electric Co.) produced an "acidic electrolyzed water" containing hypochlorous acid (HOCL), hydrochloric acid (HCL), and dissolved chlorine (Cl_2) by the electrolysis of tap water containing dissolved sodium chloride. Likewise the MIOX system produced a mixed oxidant solution (MOS) consisting of hypochlorite, chlorine dioxide, and ozone from sodium chloride and tap water (MIOX Corporation, Albuquerque, NM). Hydrogen peroxide was produced by passing electricity through water using specialized electrodes [5]. Ozone was produced by creating an electrical discharge to "super-oxygenate" ordinary tap water using a patented technology [6]. Clorox (provided by Clorox Bleach Company, 1221 Broadway, Oakland, CA, 94612) was used as a negative (no survival) control. Chlorine dioxide, produced by manually mixing patented chemicals (Doona, C. and F. Feeherry, NSRDEC), was also tested.

Table 1. Sanitizer generators make and models

Model	Company	Address	Sanitizer	Technology	Precursor
Electrocide	Electrolyzer Corporation	Woburn MA	Hypochlolous Acid (HOCl)	Electrolytic Cell	NaCl (table salt)
FC-2	Water Star Inc.	Chardon OH	Hydrogen Peroxide (H_2O_2)	Electrolytic Cell	none
Lotus	Tersano	Woodbridge VA	Ozone	Electrolytic Cell	None
SAL-40	MIOX	Albuquerque NM	MIOX	Electrolytic Cell	NaCl (table salt)
ROX-20TA-U	Hoshizaki	Peachtree City GA	Hypochlorous Acid (HOCl)	Electrolytic Cell	NaCl (table salt)

2.2 Inoculation of Surfaces

2.2.1 Stainless Steel Coupons. Custom stainless steel (type 304) coupons were the surfaces used in these experiments. The total surface area was 10.16 cm^2 (4"x 4"). The stainless steel coupons were washed and brushed clean in RBS 35 detergent (Pierce Co., Rockford, Il) in tap water at 50° C, then autoclaved in RBS 35 solution for 15 min. Each coupon was then rinsed three times with tap water, then three times with distilled water, air dried, and finally soaked in absolute ethyl alcohol and air dried. The coupons were laid in aluminum foil trays which were covered and sealed with aluminum foil. Trays and coupons were sterilized at 121° C for 30 min.

2.2.2 Test Bacteria. Three strain cocktails were prepared for *Staphylococcus aureus* (ATCC 6538, 13567, 8095) and *Escherichia coli* [ATCC 11229, 43827, 43888 (nontoxic 0157-H7)]. Each bacterial strain was activated in Trypticase Soy Yeast extract Broth (TSYEB, Difco) at 35° C by making three transfers at 20-24 h intervals. Mixed inoculums of *S. aureus* and *E coli* were prepared separately by pooling equal volumes of a 1:10 TSYEB dilution of each strain in a single test tube. Sterile Tween 80 (0.1 ml of a 1% solution) was added to 8-10 ml of culture and mixed by vortexing. The purity of all cultures was checked. The morphology, gram reaction, growth, and appearance on D/E Neutralizing agar (Difco) were also checked and documented. All stock cultures were maintained in Cystine Trypticase Agar Medium (CTA, Difco). Working cultures were maintained on Trypticase Soy Agar (TSA, Difco) for 30 days under refrigeration. At the end of 30 days a fresh TSA slant was prepared from the CTA stock culture.

2.2.3 Inoculation. Stainless steel coupons were inoculated with 0.2 ml of pooled cultures, diluted to deliver 10^7 cells, and spread evenly over the entire surface of the stainless steel coupon with a sterile spreader. Coupons were then air dried at room temperature in a laminar down-flow biological hood for 30 to 60 min. Three coupons served as subject test surfaces for the sanitizer, three coupons served as negative (no survival) controls, three coupons served as a tween-80 positive count (no kill) controls, and two coupons served as the untreated numbers control. An additional coupon inoculated with sterile distilled water served as a sterile control.

2.3 Efficacy Testing

2.3.1 Sanitizer Efficacy Test. After drying as above, all test coupons were immersed in the test sanitizer for 5 min. This contact time was selected because several investigators had reported, in the literature, lethal concentrations after 5 min for each sanitizer chosen for testing and for each of the neutralizers recommended. These literature findings are presented in Table 2. In the contact time trials the coupons were immersed for 30 sec, 1 min, 2 min, and 5 min.

Table 2. Lethal concentrations, contact times, and effective neutralizers for selected sanitizers

Sanitizer	Active Ingredient	Contact Time	Concentration (ppm)	Neutralizer	Reference
CLO_2	Cl_2	5 min	≤9.3	ST/NB [b]	2,10,11,12,13
AEW [a]	HOCL	5 min	≤47-53 FAC	ST/NB [b]	11,12,13, 14
H_2O_2	OH	5 min	>6 %	1% Na Pyruvate	11
MIOX	MOS[C]	5 min	≤200 FAC	ST/NB	11,12,13
Ozone	O_3	5 min	4.0-5.0	1% Peptone water	11

[a] Acidic electrolyzed water (FAC)
[b] Sodium thiosulfate / Neutralizing buffer
[c] Mixed oxidant solution: CL_2, O_3, and ClO_2

The requirement for an effective sanitizer on surfaces was that it produced at least a 5 log reduction within 5 min [10, 15, 16, 17]. The plan outlined in Table 2 takes into consideration the

purpose of the investigation, which was to evaluate five generators for their ability to produce sanitizer solutions effective on surfaces in the field. A fair and reasonable, fixed contact time was established that was supported by the literature and applied to all the sanitizer tested. Although it was anticipated that the concentration of each sanitizer generated may vary, it would be accepted unless it was unsafe, in which case it would be diluted. After a sanitizer / generator was tested and found to be effective, the least contact time required could then be determined, if necessary. Initial studies were conducted to determine the optimum inoculation size for the subject bacteria, drying time after inoculation of stainless steel coupons, survival of bacteria on coupons after drying in the airflow of the clean bench, and percent recovery by swabbing. Appropriate neutralizers (listed in Table 2) were added at the end of each time period to stop the sanitizer. The coupons were again air dried as above, before sampling with Hycheck DE agar contact slides (Difco).

Table 3 shows the effective concentrations of the selected sanitizers for achieving 5 log bacterial reductions within 5 min on stainless steel surfaces, as reported by several investigators. In each case a 5-min contact time was required to achieve a 5 log reduction or greater for selective microorganisms at the concentrations listed for each sanitizer. The only exception was hydrogen peroxide, which required 15 min at the concentration shown [11].

Table 3. Bacterial reductions by selected sanitizers on stainless steel surfaces within 5 min as referenced in the literature

Sanitizer	Bacteria	Concentration	Reference
AEW	*Listeria*	52.8 ppm FAC	13
CL_2	*Listeria*	100-150 ppm FAC	11, 12
CLO_2	*E. coli*	9.3 ppm	10, 12
O_3	*Listeria*	4.0 ppm	11
	Pseudomonas	2.25 ppm	18
	E .coli	2.1 ppm	18
	Pseudomonas	5.0 ppm	19
H2O2	*Listeria*	5% (15 min)	11

2.3.2 Clorox Negative Control. Three coupons were immersed for 5 min in Clorox bleach at 200 ppm free available chlorine (FAC), which is known to be bactericidal, served as negative controls (no survival). All inoculated coupons were air dried as above, before and after exposure to Clorox, which was neutralized with sodium thiosulfate after treatment.

2.3.3 Untreated Positive Count (No Kill) controls. Three inoculated coupons were immersed in 0.01% Tween 80, a non-reactive reagent with no bactericidal activity.

2.3.4 Numbers Control. To determine the number of recoverable bacteria, two coupons were inoculated, dried as above (no further treatment), and then swabbed with a Millipore Swab Test Kit (PB427). Swabbing was performed by combining recommended procedures of the Millipore Corp. and the U.S. Public Health Service [20]: five 4-in^2 (2"x2") areas of both coupons were

swabbed using a minimum of 10 strokes (about 2 linear inches each) in one direction and 10 strokes in the other direction. Initial strokes were then repeated so that the same area was covered. The swab was returned to original container of buffered solution, rotated (whip rinsed) in the diluent and excess liquid pressed out (leaving swab moist). Using the same swab, four more 2"x 2" areas were swabbed, rewetting the swab and pressing out excess liquid each time before swabbing successive areas (The total area swabbed was 16 in^2). The swab was inserted into case, vortexed for 1 min, and shaken manually 30 times through a 1-ft arc. Ten-fold dilutions were prepared and standard aerobic spread plate counts were performed [21] in Plate Count Agar (PCA, Difco).

2.3.5 Sterile Control. A single coupon inoculated with sterile distilled water served as a sterile control. It was then dried as above, before sampling with Hycheck DE agar contact slide (Difco).

2.3.6 Sampling with Hycheck Agar Contact Slides. Surviving bacteria adhering to the coupons were recovered and enumerated on Hycheck D/E neutralizing agar contact slides (Difco). Four areas of each coupon were sampled using both sides of a contact slide (two Hycheck slides per coupon). Contact Slides were incubated in an upright position at 35° C.

2.3.7 Planktonic Cells. Recovery and enumeration of the cells that did not adhere and were sloughed off the coupons into the sanitizer were accomplished with Millipore Total Count Samplers.

2.3.8 Quenching of Sanitizers. All sanitizers tested were quenched (neutralized) immediately following treatment of the inoculated stainless steel coupons, before conducting plate counts with Hycheck DE Neutralizing agar contact slides or Millipore samplers (see Table 3).

2.3.9 Chlorine Test Strips. FAC concentration of appropriate sanitizers was tested with Serim Monitor Test strips for chlorine, which were provided by Serim Research Corporation, PO Box 4002, Elkhart, IN.

2.3.10 Chlorine Dioxide Test Strips. Chlorine dioxide concentration of the manually prepared solution was measured with INSTA –Test CLO_2 test strips, provided by Lamotte, Chestertown, MD.

3. RESULTS

Of the sanitizers tested, shown in Table 4, AEW, CLO_2, MIOX, and Clorox were effective biocides at the use concentrations when tested against three strain cocktails of *S. aureus* adhered to stainless steel coupons, after 5 min contact time. Of the four sanitizers, the established five log reduction requirement was exceeded by ≥2 logs by all except the Hoshizaki AEW (≥5.1). No colonies were recovered from any of the three coupons inoculated, after sanitization. The lower, but still effective, LR by the Hoshizaki AEW was probably due to the lower FAC concentration measured which was between 25 and 50 ppm.

Table 4. Efficacy of sanitizers with *S. aureus* adhered to surface of stainless steel

	Average CFU[a] / Coupon					
Sanitizer	Before Treatment	After 5 Min	LR[b]	ppm[c]	pH	ORP[d]
AEW[e]	3.4×10^7	0.0	>7.53	88.0	3.0	1085.0
AEW[f]	1.2×10^7	94.0	>5.10	>25<50	2.93	1121.0
H_2O_2	1.02×10^7	$>5.0 \times 10^4$ [g]	<3.41	3750.0	8.0	271.0
Ozone	1.8×10^8	$>3.2 \times 10^3$ [g]	<4.62	1.02	7.0	670
Clorox	3.4×10^7	0.0	>7.53	200.0	10.1	592.0
ClO_2	1.13×10^7	0.00	>7.05	100	6.27	669
MIOX	1.40×10^7	0.0	>7.14	1000	8.9	759
None[h]	2.5×10^7	$>2.5 \times 10^4$ [g]	< 3.0	-	4.5	-
Control[i]	1.9×10^7					

[a] CFU - Colony forming unit
[b] LR – Log reduction
[c] FAC except for ozone and ClO_2
[d] ORP – Oxidation reduction potential
[e] Electrolyzer Corp.
[f] Hoshizaki Co.
[g] Estimated count
[h] Tween 80 (positive count control)
[i] Numbers control (inoculated coupon)

Although they are known to be effective sanitizers, ozone and hydrogen peroxide failed to achieve a 5 log reduction in these studies. This was undoubtedly due to low concentrations produced by the generators. Counts on Hycheck neutralization agar were too numerous to count (TNTC, >250 CFU/in^2) for both adhered and planktonic cells, indicating that the required 5 log reduction was not achieved. Hydrogen peroxide had an oxidation reduction potential (ORP) of only 271 and measured between 2500 ppm (0.25%) and 5000 ppm (0.5%), which were much below lethal levels (Tables 2 and 3). The instability of ozone was demonstrated by the reduction of the ORP from 838-836 to barely lethal levels of 662-678 (average. 670), in less than 5 min.

The purpose was to evaluate the generators as a source of effective bactericidal sanitizers for hard surfaces at whatever concentration was produced. If a sanitizer was not effective against *S. aureus* it did not meet criteria that were established and consequently was not tested against *E. coli.* Tween 80 has no active ingredients and served as a positive count control. The counts

obtained with the numbers control (untreated coupon) served to verify that bacteria did adhere to the stainless steel coupon and were recoverable. However, the numbers control was determined by swabbing the coupons, followed by standard aerobic plate counts (APCs) on Plate Count Agar (PCA, Difco); whereas, all other counts in Table 4 were obtained on Hycheck Contact Slides.

Table 5 shows the efficacy of AEW and Clorox for reducing *E. coli* adhered to stainless steel coupons after 5 min of contact time. No colonies were recovered from the coupons after treatment. Since ozone and hydrogen peroxide were not effective with *S. aureus* at the concentrations generated, they did not meet the criteria and consequently were not tested with *E.coli*. Tween 80 was the positive count control and had no bactericidal activity.

Table 5. Efficacy of selected sanitizers with *E. coli* adhered to the surface of stainless steel

Sanitizer	Average CFU[a] / Coupon Before Treatment	Average CFU[a] / Coupon After 5 Min	LR[b]	FAC ppm	pH	ORP[c]
AEW[d]	1.2×10^7	0.0	7.1	75.0	3.0	1082.0
AEW[e]	1.56×10^7	0.0	7.2	25-50	2.9	1119.0
Clorox	1.2×10^7	0.0	7.1	200.0	10.1	581.0
None[f]	1.2×10^7	$>1.2 \times 10^4$	< 3.0	-	4.5	-
Control[g]	1.6×10^5					

[a] CFU - Colony forming unit
[b] LR – Log reduction
[c] ORP – Oxidation reduction potential
[d] Electrolyzer Corp.
[e] Hoshizaki Co.
[f] Tween 80 (positive count control)
[g] Numbers control (untreated coupon)

Tables 6 and 7 show the planktonic cell counts. These were counts of non-adherent cells sloughed off the coupons and put into suspension for five min, after the coupons were immersed in the sanitizer solutions. As with the adhered cells, only AEW, ClO_2, MIOX, and Clorox were effective against planktonic *S. aureus* (Table 6).

Table 7 shows that AEW and Clorox effectively reduced planktonic *E. coli* as expected, since they were effective against the more resistant *S. aureus*. Ozone and hydrogen are not shown in Table 7 because they were not effective with *S. aureus* planktonic cells as required by criteria. MIOX and ClO_2 were not tested against *E. coli* planktonic cells for logistical reasons, so they are also not shown in Table 7.

Table 6. Efficacy of sanitizers with non-adherent (planktonic) *S. aureus* washed from inoculated stainless steel surfaces after immersion in the sanitizer solution

Sanitizer	Average CFU[a] / Coupon Before Treatment	After 5 Min	LR[b]	ppm[c]	pH	ORP[d]
AEW[e]	3.40×10^7	0.0	>7.53	88.0	3.0	1085.0
AEW[f]	1.20×10^7	167.0	>4.85	25-50	3.0	1121.0
Clorox	3.40×10^7	0.0	>7.53	200.0	10.1	592.0
H_2O_2	1.0×10^7	$>5.3 \times 10^4$ [g]	<2.30	3750.0	8.0	271.0
Ozone	1.8×10^8	$>5.0 \times 10^4$ [g]	<3.64	1.02	7.0	670
MIOX	1.40×10^7	0.0	>7.14	1000	8.9	759.0
ClO_2	1.13×10^7	0.0	>7.15	100	6.27	669
None[h]	2.50×10^7	$>2.5 \times 10^4$ [g]	< 3.0	-	4.5	-
Control[i]	1.90×10^7					

[a] CFU - Colony forming unit
[b] LR – Log reduction
[c] FAC except for ozone and ClO_2
[d] ORP – Oxidation reduction potential
[e] Electrolyzer Corp.
[f] Hoshizaki Co.
[g] Estimated count
[h] Tween 80 (positive count control)
[i] Numbers control (untreated coupon)

Table7. Efficacy of selected sanitizers with non-adherent (planktonic) *E. coli* which were washed from inoculated stainless steel surfaces after immersion in the sanitizer solution

Sanitizer	Average CFU[a] / Coupon Before Treatment	After 5 Min	LR[b]	FAC ppm	pH	ORP[c]
AEW[d]	3.40×10^7	0.0	>7.53	88.0	3.0	1085.0
AEW[e]	1.56×10^7	0.0	>7.2	25-50	2.93	1119.0
Clorox	3.40×10^7	0.0	>7.53	200.0	10.1	592.0
None[g]	2.50×10^7	$>2.5 \times 10^4$ [f]	< 3.0	0.0170	4.5	-
Control[h]	1.90×10^7					

[a] CFU – Colony forming unit
[b] LR – Log reduction
[c] ORP – Oxidation reduction potential
[d] Electrolyzer Corp.
[e] Hoshizaki Co.
[f] Estimated count
[g] Tween 80 (positive count control)
[h] Numbers control (untreated coupon)

The importance of contact time on the efficacy of AEW (Electrolyzer Co.) on stainless steel surfaces is shown in Table 8. The AEW was tested against adherent and non-adherent *S. aureus* and *E. coli* as before except that different contact time periods were investigated. The contact times were 30 sec and 1, 2, and 5 min. AEW (100 ppm FAC) achieved a 5 log reduction of adherent *S. aureus* cells, only after five min contact time, by reducing the colony count to only 13 colony forming units (CFUs). The sanitizer was effective on non-adherent (planktonic) *S.*

aureus cells after 1 min, demonstrating that cells in suspension are less resistant than adherent cells as previously reported. Both adherent and non-adherent *E. coli* were reduced to 0 CFUs after only 1 min demonstrating that *E. coli was* much less resistant to AEW than *S. aureus.*

Table 8. Efficacy of Electrolyzer AEW[a] and contact time on adherent and non-adherent bacteria inoculated onto stainless steel surfaces

	Average Colony Forming Units per Coupon[b]			
	"Adherent"		"Non-Adherent"	
Time	*S. aureus*	*E. coli*	*S. aureus*	*E.coli*
30 sec	268	43	11,000	0
1 min	160	0	0	0
2 min	173	0	0	0
5 min	13	0	0	0

[a] Average 100 ppm FAC
[b] Inoculum/coupon: 1.2 million CFUs

These studies indicated that AEW as produced by the Electrolyzer Corporation generator is effective as a hard surface sanitizer with both *S. aureus* and *E. coli* adhered to stainless steel and also with cells that became suspended in the sanitizer.

Table 9 presents another study to compare the effect of contact time of four sanitizers on stainless steel surfaces *with* adherent and non-adherent *S. aureus*. AEW from both systems, where contact time was directly compared, were similarly effective against adherent cells, and 5 min was required by both to achieve the required 5 log reduction. However, with non-adherent *S. aureus,* the Electrolyzer system was more effective than the Hoshizaki system after 1 and 5 min, probably due to the higher concentration of FAC. The results demonstrated again that attached cells are more resistant to sanitizers than the non-adherent cells. MIOX achieved greater than a 7 log reduction at all time periods tested, but the high FAC concentration (1000 ppm) was hazardous and too corrosive for sanitation. Chlorine dioxide at 100 ppm was more effective than AEW and also achieved greater than a 7 log reduction of adherent and non-adherent *S. aureus,* after only 1 min contact time. The pH was 3 for AEW, 8.9 for MIOX, and 6.3 for CLO_2. The oxidation reduction potential ranged between and 669 for CLO_2 to 1101 for AEW. All controls were effective and performed as expected.

Table 9. Efficacy of selected sanitizers and contact time on adherent and non-adherent *S. aureus* inoculated onto stainless steel surfaces

Contact time	Log Reduction							
	AEW[a] 100 ppm FAC		AEW[b] 25-50 ppm FAC		MIOX 1000 ppm FAC		ClO_2 100 ppm	
	A[c]	NA[d]	A	NA	A	NA	A	NA
30 sec	3.62	2.03	-	-	-	-	-	-
1 min	3.87	>6	4.54	3.64	>7	>7	>7	>7
2 min	3.84	>6	-	-	-	-	-	-
3 min	-	-	4.93	4.68	>7	>7	>7	>7
5 min	5.0	>6	5.10	4.85	>7	>7	>7	>7

[a] Electrolyzer AEW
[b] Hoshizaki AEW
[c] Adherent cells
[d] Non-adherent (planktonic) cells

4. DISCUSSION

The AEW produced by the two generators tested met all the requirements for a hard surface sanitizer that were established for this study. The antimicrobial mechanisms of AEW are not fully understood [14], but as reported, the presence of chlorine and a high ORP contribute to the effectiveness of the sanitizer. Hydrogen peroxide and ozone were not effective against *S. aureus* attached to stainless steel, or on non-adherent cells in suspension, after treatment for five min. The ineffective performance of the hydrogen peroxide and ozone was undoubtedly due to the low concentrations produced by the generators. As pointed out by Robbins [11] for example, hydrogen peroxide is not effective after 5 min at less than 6% and required more than a 15 min contact time (Tables 2 and 3). The ORP for hydrogen peroxide measured only 271, which is about one-third of a lethal ORP value [22]. The MIOX generator was effective but the hypochlorite measured at 1000 ppm was impractical and too hazardous for sanitation. The mixed oxidants produced (chlorine, hypochlorite, chlorine dioxide and ozone) would undoubtedly be effective at a lower recommended concentration of 200 ppm, but further testing was discontinued because the unit tested was too large and impractical for use in field kitchens.

Although CLO_2 (Doona C. and F. Feeherry, NSRDEC) was not produced by a generator, it is known to be one of the most powerful anti-microbials available [23]. As it was in this study, CLO_2 has also been reported to be more effective than chlorine at smaller dosage and reaction time. In addition, it removes flavors and odors [24]. However, CLO_2 does not have no-rinse approval for produce [24]. Safe and user friendly generators of CLO_2 are available, and two are offered by Bio-Cide International, Norman, OK.

The precedent and justification for selecting a 5-min contact time for all sanitizers tested was gleaned from the literature (Table 2). Five min was the contact time specified by a U.S. Environmental Protection Agency (EPA) proposal [15, 16] and by a European Standard [10]. To be an effective sanitizer, the requirement was that it produced at least a 3 log [15, 16] to 5 log [10] reduction within 5 min. The USFDA sanitation requirement is also 5 log [17].

While sanitizer generators may be effective for water purification, it can not be assumed that they will also be effective for sanitizing hard surfaces. It is known that biofilms on surfaces provide bacteria protection against sanitizers [9, 12, 25]. Bacteria attached to surfaces are killed by sanitizers only at concentrations orders of magnitude higher than what is required to kill planktonic cells [13]. Le Chevallier [26] found that biofilm bacteria were 150-3000 times more resistant than planktonic cells to hypochlorous acid. More recently, Robbins et al [11], found that a four-fold to sixteen-fold increase in concentration of ozone was needed to kill *Listeria* on surfaces as compared to planktonic *Listeria* cells.

A 5-min contact time would be impractical in the field and is also unnecessary because the surfaces tested will be clean and the biofilms have been removed. On a properly cleaned surface the counts will be very low, only a few/in^2 so that sanitization happens in seconds. To demonstrate the required 5 log reduction in this study, it was necessary to inoculate the test surfaces with 10^7 to 10^8 CFUs. Because bacteria are not killed instantly when exposed to a sanitizer, it was anticipated that several minutes contact would be necessary to achieve the required efficacy. This phenomenon was demonstrated with chlorine in the following example

[12]: Chlorine at 200 ppm, applied to a stainless steel surface exposing adherent *Listeria* cells, achieved a 3 to 4 log reduction in less than 1 min. Then inactivation continued at a slower rate until a 5 log reduction was achieved, but only after 5 min. More than 5 min was required when these surfaces were exposed to only 100 ppm chlorine. Inactivation of cells proceeded at a slower rate, and a detectable number of cells was still present on the stainless steel after 5 min [12]. This example and the results in Tables 8 and 9 show clearly that both sanitizer concentration and contact time were the key to determining sanitization efficiency.

Laboratory testing of the five generators has been completed. In addition, a patented and proprietary Chlorine Dioxide system developed at NSRDEC is being tested on fruit and vegetables at NSRDEC (Doona and Feeherry), and was also tested with the stainless steel coupon system reported above.

It should be noted that the sanitizer generators tested must have a water and power source available in order to be practical for use in the field.

5. CONCLUSIONS

Laboratory testing of the solutions produced by five generators previously identified and a patented and a proprietary, manual chlorine dioxide system was completed on stainless steel surfaces. AEW concentrations of 80 -100 ppm FAC and chlorine dioxide at 100 ppm were effective with both adherent and non-adherent *S. aureus* and *E. coli* bacterial cells. Such generators would provide MKT, AK, and CK with the means to produce an effective sanitizing solution in the field without re-supply. They would also eliminate the logistical problems and hazards entailed with the purchase and shipment of chemicals throughout the military supply system. The generators will require electrical power and a potable water supply. The model devised for conducting these tests performed as expected and with excellent and reproducible results. It is highly recommended for use in studies such as these because it saved considerable time, expense, and effort and the results were easily determined.

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